

## A PROBLEM IN ALGAL ECOLOGY — CONTAMINATION OF HABITATS FROM ADJACENT COMMUNITIES

Frank E. ROUND

School of Biological Sciences, University of Bristol,  
Bristol BS8 1UG, England

**ABSTRACT** — Problems associated with the contamination of distinct diatom communities by live or dead frustules (valves) from adjacent communities are discussed. The importance of the recognition and discrete sampling of the numerous microhabitats in both freshwater and marine systems is stressed. Special comments are made concerning the complexity of the epilithic flora in rivers and the "metaphyton" associated particularly with the epiphyton.

**RÉSUMÉ** — Les problèmes associés à la contamination de communautés distinctes de diatomées par des frustules (valves) morts ou vivants ■ provenance de communautés adjacentes sont discutés. L'importance de la reconnaissance et de l'échantillonnage séparé des nombreux microhabitats, aussi bien en eau douce que dans le milieu marin est souligné. Des commentaires particuliers sont faits sur la complexité de la flore épilithique des rivières et le "metaphyton" associé notamment avec l'épiphyton. (Traduit par la Rédaction)

**KEY WORDS:** Bacillariophyceae, contamination of communities, diatoms, epipelon, epiphyton, epipsammon, microalgae, plankton.

### INTRODUCTION

Unlike the situation confronting higher plant ecologists, workers in micro-algal freshwater or marine ecology are faced with admixtures of live or dead cells from several adjacent communities within their chosen micro-habitat.

Sampling of microscopic organisms — unlike higher organisms — usually involves sampling ■ sector of the physical habitat, e.g. a litre of water, an area (volume) of sediment, an area of rock or plant surface. But movement in the surrounding water deposits live (and dead) organisms from numerous niches into the chosen niche. Experience of the range of habitats enables workers to detect these and make appropriate allowances. Nevertheless, the literature abounds in records of contaminants, e.g. of benthic taxa in plankton lists and *vice versa*.

The problem is even more acute where listing or counting of unicells involves species which have cell walls which are resistant to decay, e.g. desmids, diatoms, coccolithophorids, *Pediastrum*, *Scenedesmus*, *Trachelomonas*, Dinoflagellate and Chrysophyte cysts, etc.

Few papers discuss these problems and it is only by studying the data in papers and from working continuously with samples taken from precise habitats that the problems surface. Two features strike me as major problems. The first is that precisely chosen and described habitats (other than the plankton) are rarely adequately sampled and secondly, deriving from the crude sampling the exact habitat of many microalgae is unknown — it certainly rarely appears in modern floras where one might expect this information.

Contamination (defined by the Oxford English Dictionary as “to render impure by contact or mixture”), is not easily detected in aquatic systems without complete knowledge of all the associated floras, but at least the obvious impurities can be removed, e.g. by separating silt and sand from plant surfaces and stones. At the same time planktonic species can be separated. The sediment community (epipelon) can be sampled by utilising the phototactic movement of species out of the sediments thus isolating the live cells from the multitude of dead cells. Merely studying the sediment mixes all the algal communities of the water body.

Diatoms *do* occupy distinct habitats and *do* form discrete associations but these are rarely defined since few workers make assessment of live cells, and when the sampled material is cleaned to make microscope slides/SEM preparations, all the casual cells confuse the true association.

Some aspects will now be considered in greater detail, concentrating on the diatoms.

## CONTAMINATION OF COMMUNITIES

Paper after paper concerned with diatoms in the environment\* contain lists of species which are present in small numbers. Why are they there? This is not always simple to answer, so let us consider some of the approaches. Many records are made from samples boiled in acids, thus no account can be taken of dead cells/valves swept up in the sampling. I will come to other problems of sampling later. Ecological studies ought to be based on live material, all diatoms should be identifiable by light microscopy of live material (see Cox, 1996). It is possible, though no comprehensive floras based on internal cell morphology exist and thus every worker has to make their own flora. One of the tragedies of diatom studies is the almost total lack of consideration of live cells both in ecology and taxonomy: no other algal group suffers this massive hindrance. Theoretically and often practically all diatom communities contain dead cells of the actual community (in the epilithon it is quite common to encounter samples in which almost all the cells are dead) but also varying proportions of dead cells from other communities — the former confuse the instantaneous population data — the latter are just irrelevant. E.g. in a recent study (involving planktonic diatoms of a lake), 10 out of 47 species listed were truly planktonic — it is difficult to see how this happened if sampling was in mid-lake and with uncontaminated apparatus. One could envisage situations where flocs of diatom contaminated material occurred in the open water but these should be obvious and noted as such, not recorded as plankton. The benthos is however a much larger problem. All habitats may be bathed in planktonic material which cannot be avoided by any conceivable sampling technique — the

\* (I hesitate to use the term diatom ecology — ecology is the most difficult aspect of biology, the last to be tackled only after all other aspects have been put in place, including the physics and chemistry of the environment — most so-called ecology is merely listing species).

only solution is the usually easy recognition and discarding of planktonic cells in the analysis, e.g. river epipelon is at certain times of the year overwhelmed by *Nitzschia acicularis* (Kütz.) W. Sm. deposited from the water column. But also each benthic habitat (see below) can receive cells from other benthic habitats and these must somehow be screened out. What often happens is that counts are made on acid cleaned material (it is difficult to do otherwise) and a percentage limit set to discard minor components. Strangely it seems true that contaminants, though always present, are never as abundant as one might expect from theoretical considerations! A fortunate situation. But a problem arises here since detailed sampling often reveals that minor components consistently occur in certain environments, e.g. *Navicula integra* (W. Sm.) Ralfs is never abundant in British river epipelon but at some stations low down the rivers it occurs in relatively small cell numbers and must reflect the conditions within these reaches. Only lengthy detailed sampling will reveal such critical aspects of diatom ecology. Many other rare species are, however, simply washed downstream — these can only be determined by sampling at close intervals — or preferably, devising suitable techniques for counting live cells. In general, in my experience, live cells are rarely transported into other communities in any quantity — they tend to die rapidly when removed from their intrinsic niche.

This problem is of course multiplied when mixed communities are sampled, since not only two (or more) discrete communities are recorded as one, but the contaminants of both are added in. What is a mixed community? Each worker must decide this himself, but see below.

Some habitats act as traps for casual species, e.g. mosses such as *Fontinalis* contain a mixture of species. The epipelon lives of course amidst a deposit of species from other habitats but to some extent the motile cells can be at least concentrated and removed from the sediment by various techniques, though it is difficult to make a total quantitative collection of the epipelon and at the same time eliminate contamination.

## ON TAXONOMY/ECOLOGY

It is useful when deciding on the identity and systematic position (generic) of a taxon to ask about its ecology. At the most basic level, marine or freshwater — an apparently elementary question, quite so, but nevertheless a fundamental one. But what is the proper question? I believe it is "where is the centre of distribution of the taxon in question"? Is it in water of a salinity of 0.1 to 2.0‰ (i.e. you would drink it) or is it above this and more likely approaching 35‰ (in which case you would not drink it). Arguments about the region around 2.0‰ are relevant in special areas, e.g. the Baltic coasts and it is essential in these situations to make very careful studies of the tolerance of each and every species. Here it is worth bearing in mind the single most important sentence I can find in the one and only book I know of entitled *Ökologie der Diatomeen*, i.e. Cholnoky's 1968 publication. Here he states "ecology is the study of the physiology of tolerance" — though not here in relation to salinity tolerance but the much more subtle effects of nitrogen heterotrophy and other more controversial aspects. Equally, but much simpler than in the Baltic situations, is the salinity encountered in the very common, especially but not inclusively in the semi-arid/arid regions of the land masses where evaporation produces inland saline lakes. Here the indicator species are well known and well documented.

To return to the freshwater/marine situation, an attempt was made by Pat Sims and myself to indicate the extent to which genera are exclusive to (or better have their centre

of distribution in) freshwaters or marine habitats. Since this was published in 1981 it has become increasingly clear that our earlier statement that "the exceptions to the rule" are the result of inadequate taxonomy has proved true. For example, *Navicula* now contains only freshwater genera of the old section Lineolatae, *Nitzschia* has had several sections removed thus clarifying its freshwater centre of distribution (work still remains to be done).

Within the freshwater environments a trace of salinity, purely of marine origin (e.g. sea spray) will result in the occurrence of *Ctenophora pulchella* (Grun.) Williams & Round — further evidence for its individual status outside *Synedra* which cannot tolerate saline water. Alternatively "pollution" by industrial/agricultural input of salts also results in the occurrence of *Ctenophora* but more commonly of species such as *Caloneis amphisbaena* (Bory) Cleve, *Cyclotella meneghiniana* Kütz., making an increased contribution to the flora. Natural inland salt (not necessarily NaCl) accumulation (evaporation basins), salt springs, etc. will produce floras dominated by *Anomoconeis sphaerophora* (Kütz) Pfitzer and *Cyrtocula cuspidata* (Kütz) D.G. Mann. What is the effect on the diatom physiology — is it an osmotic effect or the mixture of salts or something more specific? Every species distribution contains clues to its environmental requirements.

Drainage from road salt stored for winter de-icing can leak into acid streams and promote the growth of *Ctenophora pulchella*, e.g. at a site on the River Wye which is normally dominated by the acid-loving *Eunotia exigua*. It is however most important to note that all these species are in no sense "marine" in distribution. Examples such as this indicate a positive requirement for a particular element(s) — but for what exact purpose — experimental studies are desperately needed.

### IMPORTANCE OF MICROHABITATS

The precise place in which diatoms live is important and should be recorded very specifically. The plankton is the only habitat where this is at its simplest. In general, in freshwater a net sample taken some distance offshore will be adequate and uncontaminated. However, there are complications which should be considered. Diatoms can collect at various depths during lake stratification and in oceanic regions populations can lie on the thermocline and in the upper surface populations can be affected by insolation at the surface and/or sinking from the surface. Layering is not confined to buoyant/motile species — diurnal movements can be significant. In some situations, non-planktonic diatoms associated with zooplankton either directly attached (e.g. *Protoraphis* and *Pseudohimantidium*) or associated with mucilage aggregates (involving living organisms or as organic flocs) can support communities which are as yet relatively uninvestigated. There is still much work to do even on the apparently simple plankton.

The benthos is infinitely more complex and affords a wealth of fascinating problems. It is all too easy to assume that diatom species colonise microhabitats indiscriminately. Just because a few species "appear" to be widespread is misleading. Because the benthic habitats tend to be adjacent and form mosaic patterns of colonisable sites, great care has to be taken to sample individual microhabitats. Most diatom floras have general comments and these are often misleading. Consulting slides made from acid cleaned material is usually equally confusing though slides made from carefully sampled communities can provide reliable data on community structure. Since sampling microhabitats in nature is often difficult, let me start with one of the easiest and as is so often helpful, proceed from the simple to the complex. The epipsammon, especially the marine, is easy to sample; a tube of sand

from a beach is sufficient. This will of course be contaminated by deposited plankton but this can be washed out by shaking and settling in water (tap water will do). The removal of all organic particles is necessary since they often contain old or even viable diatoms. The sand can be treated with HCl to dissolve shell fragments and with other acids, etc. to remove the numerous epipsammic diatoms (e.g. *Anorthoneis* spp., very small *Amphora*, *Navicula*, *Cocconeis* spp.) attached to the sand grains. However, do not throw away the first washings — they often contain a motile flora (*Hantzschia* is very common) — presumably an endopelagic community as yet unworked ecologically. As with the plankton this is a simplified account. On some beaches there is a diatom flora in the surface layer of sand grains and another below the surface (endopsammic — Round, 1979). Diurnal movement of the endopelagic component is commonplace (Round & Palmer, 1967). A warning! Some beaches seem to be quite barren but this is often misleading. And as one moves into estuaries the epipelagic flora of muddy sites is mixed with the epipsammic. The epipelagic can be concentrated by placing coverglasses on the surface of the sand in a Petri dish (as can the *Hantzschia* mentioned above). To avoid contamination picked up in organic material attaching to the coverglass, these can be lain on a layer of lens tissue, or a second layer of lens tissue placed on top of a first layer can be used, but such techniques require careful study in each environment since no technique is completely reliable in harvesting every species of the motile epipelagic.

An interesting experience I had recently. Sampling a river draining carboniferous limestone — the stones were encrusted with several millimetres of blue-green algal mucilage/carbonate particles. The outside was coated with *Cymbella minuta* Hilse/ *Nitzschia* spp, internally a layer of blue-green algae was rich in dead, empty valves of *Cymbella/Nitzschia*, etc. but was also rich in live *Achnanthes* spp. and a lower layer was devoid of diatoms as was the final layer adjacent to the stone surface. This was merely a casual observation and requires much more accurate detailed sampling.

The plankton, epipsammon and epipelagic have been dealt with above and all that remains is the epiphyton and epilithon. Both these are complex and collections from the field are rarely pure. The epiphyton can be observed directly and live cells counted. Higher plants, mosses and liverworts can be contaminated by diatoms disturbed by currents, etc.; flocculent material can be deposited — all this can be washed off, collected and assessed and the clean plant material treated to remove the attached epiphytic community (some loosely attached epiphytes may wash off with contaminants and care must be taken to remove the firmly attached adnate species such as *Cocconeis* — the raphid valves are often glued tightly to leaf surfaces and methods must be devised to cope with counting — there are few short cuts!! A more critical problem arises where the plant surfaces are colonised by epiphytic filamentous algae, e.g. *Oedogonium*, which may itself be colonised by *Cocconeis pediculus* Ehr. when the host Angiosperm is colonised by *C. placentula* Ehr. Large seaweeds are a much greater problem — since complex communities of filamentous and thalloid species often grow on the larger host plant — they simply have to be dissected out under a binocular microscope and some loss/contamination is difficult to avoid — careful recording of live diatoms on the various epiphytes can at least give initial information on distribution — there is absolutely no doubt that specific floras are involved (e.g. Harper & Garbary, 1994 discovered *Podocystis adriatica* (Kütz.) Ralfs on the red alga *Heterosiphonia crispella* (C. Ag.) Wynne, 1995) and also that some marine algae are devoid of diatom epiphytes. A simple freshwater example of the care needed is the fact that the undersurface of *Lemna* leaves support *Achnanthes hungarica* (Grun.) in Cl. & Grun. but the roots do not. Why is *A. hungarica* reported as confined to *Lemna*? In fact, it is not — only recently did I think to sample *Azolla* fronds and it exists there too — but why only on the small floating plants?

The epilithon is much more complex and most papers usually purporting to relate the diatom epilithon to levels of pollution, etc. are hopelessly confused. Firstly there is a range of stones in rivers from clean to complexly colonised, *i.e.* from "primary" sites to "climax" communities. The stones can be devoid of deposited sediment or coated with variable layers — on and in this a proportion of the river epipelon can grow, *i.e.* an epipelon can simply exist on a stone surface above the epilithic community. However, a word of warning — in my experience, only a proportion of the epipellic species grow in profusion on the silted stone surfaces, so here there is some selective mechanism operating. The epilithon should be thought of in terms of a forest often embedded in mucilage. The "trees" are stalked species of *Cymbella*/*Gomphonema*, the "shrubs" of stalked *Achnanthes*, the "herbs" of adnate species, *Epithemia*/*Cocconeis*, etc. How do we separate these? First wash off the epipelon, gently brush off the "trees", layer by layer (!), finally scrape off the adnate species with a razor blade. Often the layers are extremely thick and in alkaline situations complicated by calcareous deposits and other microscopic algae. Worse is to come! Many epilithic samples are further confused — the epilithon can and frequently does include attached macroalgae, *e.g.* *Cladophora*, *Oedogonium*, *Lemanea*, etc. — all with their own specific epiphytes. These can be removed but often the basal branching system and short upright branches remain and these are the oldest regions and often thickly coated with epiphytic (*not* epilithic) diatoms — a major species being *Rhoticosphenia curvata* (Kütz.) Grun. and I am not sure whether this taxon ever grows on stone surfaces. Worse still is to come. The most difficult and therefore virtually unknown diatom flora is that of solid rock surfaces in waters of all types — if they are exposed, then at least they can be scraped with a sharp blade — collecting the scraping is difficult. What is the answer? Certainly not artificial substrata unless one is interested in fouling of unnatural surfaces. The aim of the ecologist is to describe and determine the functioning of *natural* communities. There is no doubt that they are more complex than I have described in this short survey and they are infinitely more complex than the artificial assemblage on glass slides — diatom ecology is in its infancy — in fact, as a diatom ecologist myself, I would say it does not yet exist apart from a few studies. Is it so complex that synecology should give way to autecology? — certainly the latter approach is easier and can be achieved without too much taxonomic complexity.

But the future is not all gloom, not at all. The field is completely open — 99% of the work remains to be done. Research students should ignore the system which makes them perform literature surveys for months before commencing work — a sure way to get onto the wrong track or give up altogether. Better — imagine you are a diatom, and look at the problem through the eyes of a diatom and devise your sampling, etc. to answer the question you ask of the community.

## THE METAPHYTON

In a classic but sadly neglected study (Behre, 1956), the term "metaphyton" was coined for the loose flocculent community living amongst algae, especially prominent in the mucilaginous flocculent material around the stems of *Equisetum*, *Phragmites*, etc. in ponds and lakes. This is an actively growing, specific flora forming a self-standing algal association, though it may harbour some contaminants — a feature which requires further study. There is a possibility that a metaphyton occurs also in the epipelon and epiphyton of

rivers, e.g. extensive sampling of these communities over several years has revealed a "contaminant" flora of motile (*i.e.* usually epipelagic) diatoms in both communities. But it is a striking feature that the majority of epipelagic species (e.g. *Navicula capitata* Ehr., *Sellaphora pupula* (Kütz.) Mereschk., *Placoneis elginensis* (Greg.) Cox, *Surirella brebissonii* Kram. & Lange-Bertalot, *Stauroneis smithii* Grun., *Neidium dubium* (Ehr.) Cl., *Caloneis silicula* (Ehr.) Cl., *Amphora ovalis* Kütz., *Nitzschia sigmaidea* (Nitzsch.) W. Sm. to name just obvious easily identifiable forms), are never found in the epiphyton but *Navicula tripunctata* (O.F. Müll.) Bory and *N. lanceolata* (Ag.) Ehr. are commonly found. At first I believed these latter two taxa were contaminants derived from the epipelon but further analysis showed that *N. tripunctata* is only a minor component of the epipelon (though always recorded at the majority of sites), and therefore I am forced to conclude that its favoured habitat is the epiphyton or, since it is motile, perhaps forming a metaphytonic component together with a few other motile *Navicula* species. Since all the dominants in the epiphyton are stalked or attached by mucilage pads and therefore non-motile, it has always been my assumption that any motile pennate diatoms found in epiphytic and even in epilithic communities were contaminants, but perhaps this view has to be revised. *Navicula lanceolata* remains a problem, however, since it is obviously a dominant, motile diatom in the epipelon and it is well represented in numerous samples of epiphyton and epilithon — though washing of plants and stones does reduce its presence. There is a need to check unsilted epiphyton and epilithon where it is less likely to be present. At the moment my inclination is to favour the epipelon as its natural habitat. This data does not mean that care must not be relaxed when studying the attached communities and even greater attention must be paid to distinguishing between contaminants and possible metaphyton.

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